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Conf rmational Changes Induced in th Human Immunodefici ncy Virus Envelope Glycoprotein by Soluble CD4 Binding

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Summary

The human immunodeficiency virus (HIV) binds to the surface of T lymphocytes and other cells of the immune system via a high affinity interaction between CD4 and the HIV outer envelope glycoprotein, gp120. By analogy with certain other enveloped viruses, receptor binding by HIV may be followed by exposure of the hydrophobic NH_2 terminus of its transmembrane glycoprotein, gp41, and fusion of the virus and cell membranes. A similar sequence of events is thought to take place between HIV-infected and uninfected $CD4^+$ cells, resulting in their fusion to form syncytia. In this study, we have used a soluble, recombinant form of CD4 (sCD4) to model events taking place after receptor binding by the HIV envelope glycoproteins. We demonstrate that the complexing of sCD4 with gp120 induces conformational changes within envelope glycoprotein oligomers. This was measured on HIV-1-infected cells by the increased binding of antibodies to the gp120/V3 loops, and on the surface of virions by increased cleavage of this loop by an exogenous proteinase. At 37°C, these conformational changes are coordinate with the dissociation of gp120/sCD4 complexes from gp41, and the increased exposure of gp41 epitopes. At 4°C, gp120 dissociation from the cell surface does not occur, but increased exposure of both gp120/V3 and gp41 epitopes is detected. We propose that these events occurring after CD4 binding are integral components of the membrane fusion reaction between HIV or HIV-infected cells and $CD4^+$ cells.

The CD4 molecule is found predominantly on the surface of T lymphocytes, where it functions as a coreceptor for the T cell antigen receptor complex (1). It has been subverted by the human and simian immunodeficiency viruses (HIV and SIV, respectively)[1] as their primary receptor (2). CD4 is a member of the Ig superfamily (3), with a structure consisting of four extracellular Ig-like domains anchored to the membrane by a hydrophobic segment, and followed by a short cytoplasmic region (4), which interacts with $p56^{lck}$, an *src*-related tyrosine kinase (1). The high affinity binding site for the HIV outer envelope glycoprotein, gp120, is centered around a stretch of ~12 amino acids in the first domain of CD4, a region analogous to the CDR-2 loop of an Ig κ light chain variable domain (5–10). Several recent studies have demonstrated that CD4 may have a more complex role in initiating HIV infection of $CD4^+$ cells than simply binding virus to the cell surface. The CDR-3-like loop of the first domain of CD4 has been implicated in a step distinct from virus binding, which is important for membrane fusion (11, 12), and CD4 antibodies have been characterized that prevent virus infection and fusion of $CD4^+$ cells without inhibiting virus binding (13, 14).

HIV entry into $CD4^+$ cells is via a pH-independent pathway, mediated in most circumstances by direct fusion of the virus envelope with the cell membrane (15, 16), although fusion in endosomes has also been reported (17, 18). The formation of giant cells (syncytia) by membrane fusion between $CD4^+$ and HIV envelope–expressing cells is probably by a mechanism analogous to virus-cell fusion (19–22). The molecular interactions taking place after CD4 binding that result in membrane fusion are obscure. However, a soluble form of recombinant CD4 (sCD4) has been shown to induce the dissociation of gp120 from gp41 on the surface of HIV virions or envelope glycoprotein–expressing cells (23–26). Although there is no direct evidence linking this observation with priming of a fusogenic mechanism, it may be that the exposure of certain regions of gp41 after the binding of gp120 to CD4 is an essential step in initiating fusion. Such a mechanism would be analogous to the exposure of the fusogenic

[1] *Abbreviations used in this paper:* sCD4, soluble CD4; SIV, Simian immunodeficiency virus; WM, wash medium.

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peptides of orthomyxoviruses subsequent to a pH-induced conformational change in the outer envelope glycoprotein (27, 28). Additional evidence supporting this hypothesis comes from observations that at sub-inhibitory concentrations, sCD4 increases the infectivity of SIV and HIV-2, and enhances fusion of SIV- or HIV-2-infected cells with $CD4^+$ cells (29–31; and P. Clapham, personal communication). Thus, the complexing of CD4 to the HIV/SIV envelope glycoproteins may directly activate the fusogenic mechanism of gp41, the final stage of which probably involves insertion of the hydrophobic NH_2 terminus of gp41 into the host cell membrane (32–36).

In this study, we have used sCD4 to model events taking place when cell membrane–anchored CD4 interacts with HIV-1 envelope glycoproteins. We report that sCD4 binding induces conformational changes within envelope glycoprotein oligomers on HIV virions and HIV-infected cells, resulting in the increased exposure of regions of gp41.

Materials and Methods

Monoclonal Antibodies. The mAb Q4120 was prepared in our laboratory, and reacts with the first domain of CD4 (14). L120 was from D. Buck (Becton Dickinson Monoclonal Center Inc., San Jose, CA) and binds to domain 4 of CD4 (14). mAbs 9284 (37) and 110.5 (38) react with different epitopes on the V3 loop of HTLV-IIIB/gp120, and were obtained from DuPont Inc. (Wilmington, DE) and Genetic Systems Inc. (Seattle, WA), respectively. The polyclonal anti-gp120 antiserum ABT 301 was from American Biotechnology Inc. (Cambridge, MA). D7324 was from Aalto Bioreagents (Dublin, Ireland) and is an affinity-purified sheep antiserum to a 15–amino acid peptide from the conserved COOH terminus of gp120 isolate LAV-1 (39). The human mAbs 50-69 and 98-6 were from M. Gurney and S. Zolla Pazner (Veterans Administration Association, New York) (40), and have been epitope mapped by direct binding to synthetic peptides synthesized from gp41 of HIV-1/IIIB (M.K. Gorny and S. Zolla-Pazner, personal communication; see Table 1).

HIV-infected and Uninfected Cell Lines. The human T cell lines H9 and c8166 (from M. Popovic and R. Gallo, National Institutes of Health, Bethesda, MD) and H9 cells infected with HIV-1 isolate HTLV-IIIB clone HXB2 (from R. Gallo) were grown in RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 10% FCS (growth medium).

sCD4 Treatment Antibody Staining, and Analysis of Cells. H9 cells persistently infected with the HXB2 molecular clone of the IIIB isolate were washed twice in RPMI/2% FCS wash medium (WM), and resuspended at a concentration of 2×10^7 cells/ml. 50 μl of the appropriate concentration of sCD4 (from Smith, Kline, and Beecham, King of Prussia, PA) (41) was added to 50 μl of the cell suspension. After 2 h or the appropriate length of time, the cells were pelleted. The supernatants (100 μl) were removed, spun through Spin-X 0.22-μm filters (Costar, Cambridge, MA), mixed with 10 μl of a 10% solution of NP40 in PBS-A, and frozen at −20°C until assay. The cell pellets were washed in 1 ml of WM, resuspended in 100 μl of the same buffer at 4°C, and 10 μl of cell suspension (10^5 cells) was added to 10 μl of each antibody. All antibodies had been previously titrated and were used at saturating concentrations. After a 30-min incubation, the cells were washed twice in WM, then 50 μl of a dilution of the appropriate FITC-labeled second layer antibody (either anti-mouse polyvalent Igs, or anti-human IgG (Sigma Chemical Co., Poole, Dorset, UK) in WM was added. After 30 min, the cells were washed twice in PBS-A/2% FCS, then fixed overnight in 200 μl of a 1% solution of formaldehyde in PBS-A. The fixed cells were analyzed by flow cytometry on a FACScan (Becton Dickinson & Co., Mountain View, CA) using Consort 30 software. All antibody staining procedures were carried out on ice. For some experiments, the sCD4 treatment was done at 37°C, for others, at 4°C. Where the results are expressed as the mean fluorescence intensity, the background staining (second layer only) has been subtracted.

ELISA for Detection of Soluble gp120 and p24. Soluble gp120 and p24 in NP-40-inactivated cell supernatants were detected by ELISA as previously described (24, 39, 42).

Analysis of gp120/V3 Loop Cleavage by Western Blotting. Virus (HIV-1/IIIB), prepared as described previously (42), was analyzed by a combination of S-1000 gel exclusion chromatography and twin-site ELISA (24, 25, 42). The total gp120 content of the culture was 60 ng/ml, of which ~55% was present as virion-bound glycoprotein, the rest as soluble gp120. An aliquot of this virus stock (10 μl) containing 600 pg of viral gp120 was incubated for 2 h at 37°C with or without 20 μg/ml of sCD4 and/or a 1:20 dilution of mAb 110.5 ascitic fluid. After a further 2 h of incubation with 100 μg/ml of bovine thrombin (Boehringer Mannheim Biochemicals, Mannheim, Germany), the reactions were terminated by boiling with 10 μl of SDS sample buffer containing 2-ME. Proteins were fractionated by electrophoresis on a 10% reducing SDS-polyacrylamide gel, then electrophoretically transferred onto nitrocellulose (Schleicher & Schuell, Inc., Keene, NH) using a dry blotting apparatus (Sartorius GmbH, Goettingen, Germany). The filter was blocked overnight with TBS containing 20% FCS, 2% skimmed milk powder (Cadbury's Marvel), and 0.5% Tween 20, then incubated for 2 h in the same buffer containing a 1:2,000 dilution of rabbit anti-gp120 (IIIB) antiserum R1/87 (a gift from R.S. Daniels and J.J. Skehel, MRC Research Centre, Mill Hill, UK). After extensive washing of the filters in TBS/0.1% Tween 20, bound antibodies were detected with horseradish peroxidase–labeled sheep anti-rabbit Ig (Seralab, Crawley, UK) and the ECL fluorometric system (Amersham International, Amersham, UK).

Analysis of gp120/V3 Loop Cleavage by Immunoassay. Virus stock (25 μl, as above) containing 1.5 ng of viral gp120 was incubated for 2 h at 37°C with a range of concentrations of sCD4 or preformed sCD4-mAb complexes (see text) before addition of bovine thrombin (100 μg/ml) to some samples for a further 1 h at 37°C (or in some experiments for various times and temperatures; see text). The reactions were terminated by addition of Empigen detergent to a final concentration of 1% (43). The extracts were incubated with plastic-adsorbed antibody D7324 to capture gp120 into the solid phase, then reacted with mAb 110.5 as described previously (43). This antibody binds across the site of thrombin cleavage in the V3 loop and fails to bind to cleaved gp120 (43). Consequently, cleavage is detected by a reduction in 110.5 binding. Bound antibody was detected with alkaline phosphatase–labeled rabbit anti-mouse Ig (Dakopatts, High Wycombe, UK) and the AMPAK system (Novo Nordisk, Cambridge, UK) as described previously (39, 43).

Results

sCD4 Binding to HIV-infected Cells Induces gp120 Dissociation from gp41 and Exposes gp41 Epitopes. In this study, we have used sCD4 to mimic the interaction of cell-anchored CD4 with HIV envelope glycoproteins expressed on the surface of HIV-infected cells. We measured the binding of sCD4 to the cell surface, and the exposure of gp120 and gp41 epi-

Table 1. *Antibodies Used in this Study*

Reagent	Origin	Antigen	Epitope	Reference
L120	Mouse	CD4	Domain 4	14
Q4120	Mouse	CD4	Domain 1	14
9284	Mouse	gp120	302–312* (V3 loop)	37
110.5	Mouse	gp120	307–317* (V3 loop)	38
D7324	Sheep	gp120	COOH terminus	39
ABT 301	Rabbit	gp120	Polyvalent	
50-69	Human	gp41	579–613	40
98-6	Human	gp41	644–663	40

* Indicates amino acid residue numbers in minimum defined epitope of HIV-1 clone HXB2 from the Los Alamos database.

topes using mAbs to these proteins in conjunction with immunofluorescent staining and flow cytometry. In initial experiments, 10 μg/ml sCD4 was reacted with HIV-infected cells at 37°C for 2 h before mAb staining. The formation of sCD4/gp120 complexes was monitored by the binding of mAb L120 to the fourth domain of CD4 (Table 1 and Fig. 1 *a*). Before addition of sCD4 to the cells, L120 staining was at background levels, a consequence of CD4 downmodulation by HIV infection (19). The addition of sCD4 increased L120 reactivity by about fivefold over background (Fig. 1 *a*). As with L120, there was no significant binding of Q4120 to the cell surface before sCD4 addition. However, unlike L120, the addition of sCD4 to the cells did not significantly increase Q4120 reactivity (Fig. 1 *b*). Since the epitope for this mAb on domain 1 of CD4 is masked in the CD4/gp120 complex (14), this experiment demonstrates that sCD4 is bound to the cell by domain 1, implying that it interacts specifically with gp120 rather than nonspecifically with the cell surface.

The polyvalent anti-gp120 antiserum ABT 301 reacted with gp120 on the surface of the HIV-infected cells (Fig. 1 *c*). A small (2–10% in five separate experiments) decrease was observed after the addition of sCD4, suggesting that a minor fraction of the total gp120 was lost from the cell surface on CD4 binding (see Fig. 2 *c* and Discussion). Similar data were obtained with the D7324 antiserum to the COOH terminus of gp120 (data not shown), although this antibody stained poorly, implying that the epitope was mostly obscured on gp120 in its cell surface configuration. Before the addition of sCD4, the gp120/V3 loop mAb 9284 reacted strongly with the cells. After incubation with sCD4, however, 9284 staining decreased by about twofold (Fig. 1 *d*). Very similar results were obtained with another gp120/V3 loop mAb, 110.5, reactive with an adjacent but distinct epitope (data not shown). Two human mAbs that are specific for gp41 (50-69 and 98-6) stained the cells in the absence of sCD4, and showed a substantial increase in reactivity after the addition of sCD4 (Fig.

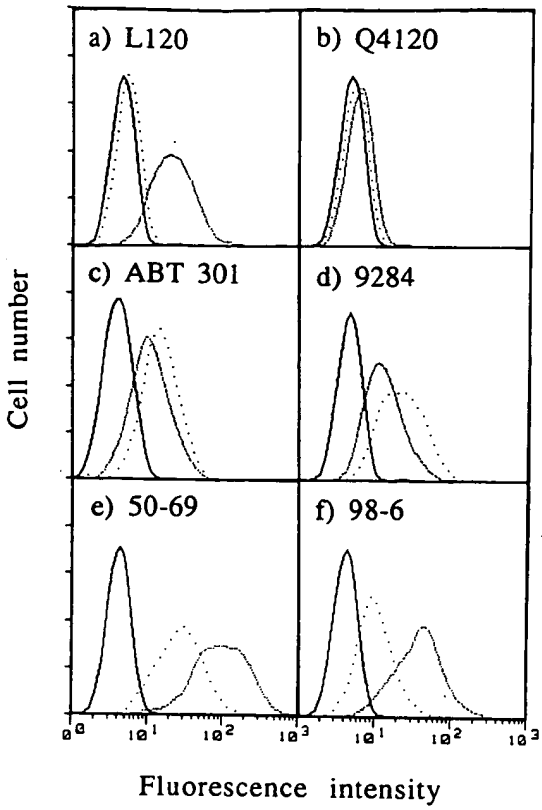

Figure 1. HIV-infected H9 cells, treated or untreated with sCD4 for 2 h at 37°C were stained with some of the antibodies listed in Table 1 by indirect immunofluorescence, and analyzed by flow cytometry. The results are expressed as overlayed histograms: (——) background staining with the FITC-labeled second layer only; (· · · · · ·) specific staining of HIV-1/HXB2 infected H9 cells untreated with sCD4; and (·····) specific staining of infected H9 cells treated with sCD4.

1, *e* and *f*). mAb 50-69 stained more intensely than 98-6 both before and after sCD4 binding.

To characterize further these observations, we investigated the effect of sCD4 concentration on the staining of gp120 and the exposure of gp41 epitopes. Concentrations of sCD4 ranging from 3.2 ng/ml to 50 μg/ml were added to infected cells for 2 h at 37°C, and the cells were stained as before. As demonstrated by L120 staining, sCD4 binding increased from virtually undetectable at the lowest sCD4 concentration, to maximal at 1 μg/ml (Fig. 2 *a*). Staining of gp120 with the V3 loop mAb 9284 initially rose as the sCD4 titer increased, was maximal at ~0.5 μg/ml of sCD4, then fell to about half the starting level (Fig. 2 *a*), consistent with the results described in Fig. 1 *d*. In contrast to the biphasic dose-response curve for V3 loop staining, the reactivity of gp41 mAbs 50-69 and 98-6 increased substantially (about fivefold and sevenfold, respectively) between 0.1 and 2 μg/ml of sCD4 (Fig. 2 *b*), and did not then decrease.

Previous studies have shown that sCD4 induces gp120 dissociation from gp41 (23–26). In the current study, the con-

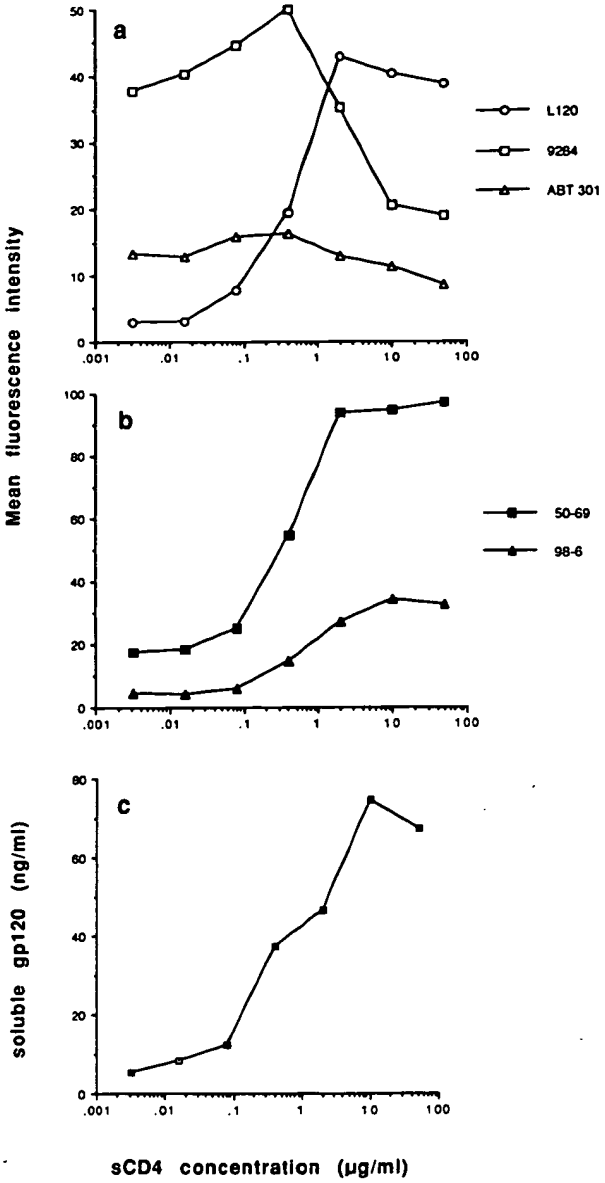

Figure 2. (*a* and *b*) HIV-1/HXB2-infected H9 cells were treated with varying concentrations of sCD4 for 2 h at 37°C before indirect immunofluorescence staining and analysis by flow cytometry. (*c*) The concentration of soluble gp120 released into the supernatant of the sCD4-treated cells stained in *a* and *b*, as measured by ELISA.

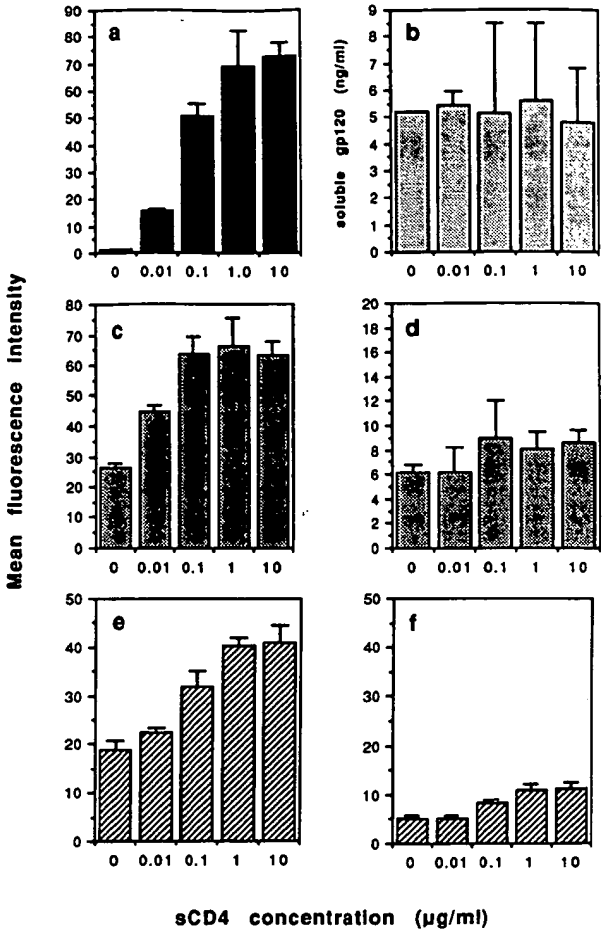

Figure 3. HIV-infected H9 cells, treated or untreated with sCD4 for 2 h at 4°C were stained with various antibodies by indirect immunofluorescent staining before analysis by flow cytometry. (*a*) The binding of CD4 mAb L120, and represents the amount of sCD4 bound to the cell. (*b*) The quantity of soluble gp120 released into the supernatant from the same cells. (*c–f*) Staining with the following antibodies: (*c*), 9284 (gp120/V3 loop); *d*, D7324 (gp120/COOH terminus); *e*, 50-69 (gp41); *f*, 98-6 (gp41). Data are the mean of triplicate experiments, error bars are ± 1 SD.

centration of free gp120 in the supernatant increased with the amount of sCD4 bound to the cell (Fig. 2 *c*). To eliminate the possibility that the increase in cell-free gp120 reflected sCD4-induced release of virions from the cell surface, we measured the amount of p24 in the supernatants. There was no significant increase in the amount of soluble p24 at any sCD4 concentration (results not shown).

sCD4 Binding to gp120 on HIV-infected Cells at 4°C Induces Modulation of Antibody Epitopes on gp120 and gp41 in the Absence of gp120 Dissociation. Because sCD4 binding causes gp120 to dissociate from gp41 at 37°C, analysis of the conformational changes is complicated. However, at 4°C, the sCD4-gp120-gp41 complex is stable (25). We used this observation to investigate whether the binding of sCD4 to gp120 on the surface of HIV-infected cells induced epitope modulation in the virus envelope glycoproteins in the absence of gp120 dissociation. The cells were saturated with sCD4 at 1 μg/ml (Fig. 3 *a*), but there was no significant dissociation of gp120/sCD4 complexes even at 10 μg/ml (Fig. 3 *b*). Thus, any modulation of HIV envelope epitopes induced by sCD4 is independent of gp120 dissociation. Staining of gp120 with mAb 9284 (Fig. 3 *c*) increased coordinately with sCD4 binding, reaching a maximum value of about twofold which was achieved in the absence of sCD4. The same increase was con-

sistently observed with 9284 in a number of experiments, and also with the 110.5 mAb (results not shown). Therefore, twice the number of gp120/V3 loop epitopes are available for antibody binding in the presence of complexed sCD4. Staining with the polyvalent anti-gp120 antiserum also increased in intensity after sCD4 treatment (results not shown), suggesting either that this antiserum has substantial reactivity with the V3 loop, or that regions of gp120 additional to the V3 loop become more accessible to antibody binding, or both. In contrast to this, however, significant modulation of D7324 staining was not observed after sCD4 complexing, demonstrating that the conformational changes were not induced globally (Fig. 3 *d*). Both of the gp41 mAbs, 50-69 and 98-6, stained the untreated infected cells at 4°C. After addition of sCD4, the staining for both mAbs increased by twofold, indicating a doubling in the number of exposed gp41 epitopes in the absence of gp120 dissociation (Fig. 3, *e* and *f*). Note that the increase at 37°C, where gp120 dissociation occurs, was considerably greater (Fig. 2 *b*).

sCD4 Binding to gp120 on Virions Increases the Exposure of the gp120/V3 Loop to Proteolytic Cleavage. Using mAbs and flow cytometry, we have demonstrated that sCD4 binding induces epitope modulation in the V3 loop of gp120 on the surface of infected cells. To confirm that similar changes take place on mature HIV virions, we monitored V3 loop exposure by its susceptibility to cleavage by an endogenous proteinase, thrombin (43). In the sample of virus that we analyzed (HIV-1/IIIB), approximately half of the gp120 present was on virions, the rest was soluble protein. At 37°C, sCD4 addition caused a major proportion of the virion gp120 to dissociate into the medium (24, 25). This was unaffected by thrombin. Thus, after sequential 2-h incubations at 37°C with or without 50 μg/ml sCD4 and 250 μg/ml thrombin added in either order, the percentages of total gp120 that were virion bound were: virus alone, 57 ± 6%; virus + thrombin, 59 ± 4%; virus + sCD4, 24 ± 4%; virus + sCD4 + thrombin, 18 ± 3%; virus + thrombin + sCD4, 20 ± 2%. At 4°C, sCD4 caused no significant loss of gp120 from the virions within 2 h, and there was only a 10% reduction in the amount of virion bound gp120 after 10 h (results not shown).

The basal rate of viral gp120 cleavage was low in the presence of thrombin alone, but was greatly enhanced by the addition of sCD4 concentrations from 0.1 to 30 μg/ml (Fig. 4 *a*). The sCD4-enhanced cleavage was blocked by prior addition of CD4 mAb Q4120, which competitively inhibits gp120 binding to CD4 (14), but was unaffected by CD4 mAb Q425, which inhibits HIV infection and syncytium formation at a step after gp120 binding (14). Thus, the effect was not due to a contaminant proteinase in the sCD4 preparation, but was a consequence of specific sCD4 binding. The potentiating effect of sCD4 on V3 loop cleavage was confirmed by Western blotting; thrombin cleavage of gp120 to 70- and 50-kD fragments was greater in the presence of sCD4 than in its absence, and was completely inhibited by prior binding of the HIV-neutralizing mAb 110.5 across the cleavage site (Fig. 4 *b*). In addition, the rate of virion gp120 cleavage was increased substantially in the presence of sCD4. It has been shown elsewhere that thrombin cuts gp120 uniquely between Arg-315 and Ala-316 in the V3 loop (43, 54).

Both virion-bound and soluble gp120 are present in the virus preparation. In principle, both are susceptible to proteolysis by thrombin. However, when virions were separated from soluble gp120 by gel exclusion chromatography (24, 42), then treated with thrombin (100 μg/ml) for 2 h at 37°C, there was no significant loss of 110.5 binding to gp120 mea-

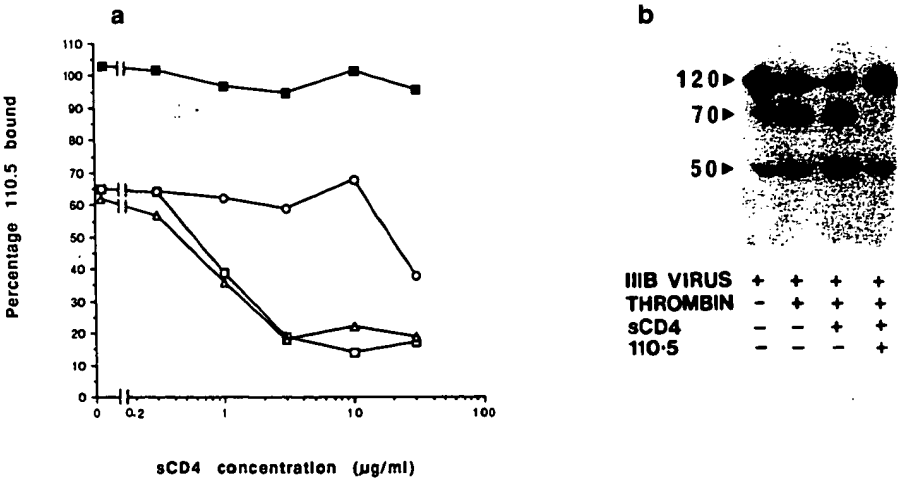

Figure 4. (*a*) Supernatant containing HIV-1/IIIB was incubated with a range of sCD4 concentrations (□) or sCD4 preincubated with the CD4 domain 1 mAb Q4120 (○) or CD4 domain 3 mAb Q425 (△) for 2 h at 37°C, followed by the addition of bovine thrombin, or without thrombin but with sCD4 (■) for a further 1 h. After solubilization of the virions in detergent, gp120 was captured to the solid phase, and the binding of gp120/V3 loop mAb 110.5 was monitored by ELISA. Since mAb 110.5 binds across the site of thrombin cleavage and cannot bind to cleaved gp120, cleavage is detected by a reduction in 110.5 binding. (*b*) Supernatant containing HIV-1/IIIB was incubated at 37°C for 2 h with or without sCD4 and/or mAb 110.5, then with thrombin for a further 2 h at 37°C. The virions were solubilized in SDS sample buffer, fractionated by polyacrylamide gel electrophoresis and analyzed by Western blotting. The figures on the left side of the blot represent molecular masses in kilodaltons.

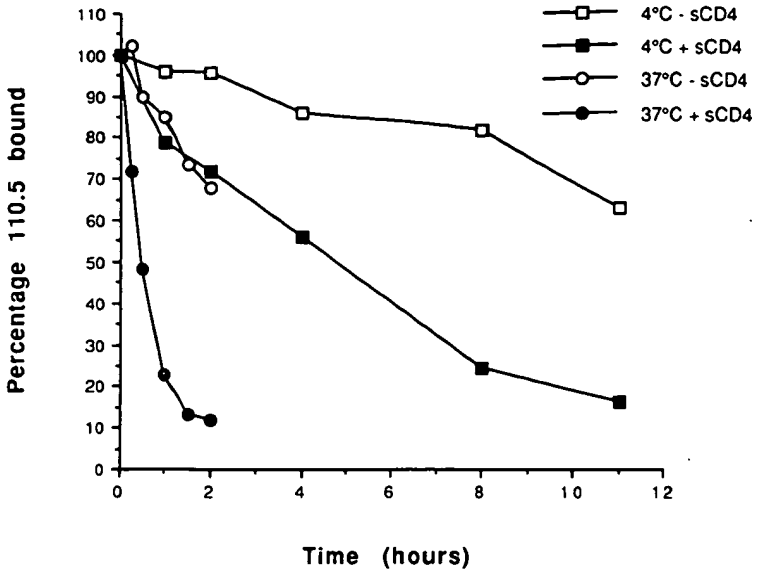

Figure 5. HIV-1/IIIB-containing supernatant was incubated with or without 50 μg/ml of sCD4 for 2 h at either 4°C or 37°C, then thrombin added at the same temperatures for various times before solubilization of virus and detection of cleaved gp120, as described for Fig. 4 *a*.

sured in a subsequent immunoassay (results not shown). On virions, gp120 is therefore resistant to thrombin cleavage. Thus, the enhancement by sCD4 of thrombin cleavage of viral gp120 might occur either by sCD4-induced conformational changes in gp120 that increase the exposure of the V3 loop to proteinases (as for recombinant gp120; 43) or by sCD4-induced loss of gp120 from the virions (24, 25) increasing the concentration of soluble gp120 accessible to the proteinase, or by a combination of these mechanisms. To address this, we carried out a thrombin cleavage experiment at 4°C, in which sCD4 binds to virions but does not cause gp120 dissociation (25). Under these conditions, sCD4 caused a clear enhancement in the rate of cleavage of gp120, and eventually most of the virion gp120 was cleaved despite about half remaining on the virion. (Fig. 5). Therefore, irrespective of any effect of sCD4-induced increase in the concentration of soluble gp120 at 37°C, sCD4 binding to gp120 on the virion surface must cause conformational changes in the viral envelope that increase the accessibility of gp120 to an endogenous proteinase. In the absence of sCD4, gp120 on the surface of the virion is not significantly cleaved by thrombin: the tertiary/quaternary structure of the envelope glycoprotein must preclude binding of this proteinase.

Discussion

Using sCD4 to mimic cell surface CD4, we have demonstrated that sCD4 complexing with the HIV envelope glycoproteins results in the modulation of antibody epitopes on gp120 and gp41, and modulation of a proteolytic cleavage site on gp120.

The data that we have obtained at 37°C are complex, since there are two phenomena occurring simultaneously: conformational changes within an envelope glycoprotein oligomer leading to increased exposure of certain epitopes, and shedding of gp120 from the cell surface. At low sCD4 concentrations, the increase in gp120/V3 loop mAb and polyvalent antiserum binding to the cells is probably due to the epitope modulation seen clearly at 4°C. The decrease in gp120 staining with higher concentrations of sCD4 probably represents gp120 loss superimposed onto the epitope modulation. The decreased staining observed with the V3 loop mAbs at 37°C was consistently greater than that with polyvalent antisera: this may represent the labeling of a large proportion of nonfunctional gp120 molecules by the polyvalent reagents, or the masking of V3 loop epitopes in addition to gp120 shedding.

At 4°C, in the absence of gp120 dissociation from gp41, the increased binding of two mAbs to different epitopes of the V3 loop of gp120, and the enhanced cleavage of this region by a proteinase, strongly suggest that this loop is more exposed after sCD4 binding. This is in accord with results obtained from proteinase cleavage of recombinant, soluble gp120 in a previous study (43). In contrast to the results obtained at 37°C, no decrease in V3 loop staining was observed at higher concentrations of sCD4 at 4°C. The increase in binding of V3 loop mAbs at 4°C was consistently found to be about twice that in the presence of saturating concentrations of sCD4 than in its absence. This approximate doubling of gp120/V3 loop mAb staining was coordinate with a twofold increase in the exposure of gp41 epitopes observed under the same conditions. Several reports describe the organization of HIV envelope glycoproteins into oligomers: the bulk of the evidence suggests that the mature structure is a tetramer (44–46), probably comprising two dimeric subunits (46). Our data may therefore represent the induction of a conformational change within gp120/gp41 oligomers, a proposal consistent with our previous report of the dynamics of sCD4-induced gp120 dissociation from gp41 (25). For example, two gp120 molecules may interact within a dimer in a nonsymmetric arrangement where the V3 loop on one mol-

ecule is exposed, but on the other, hidden. Receptor occupation of the gp120 binding regions may flip the second loop into an exposed state, destabilizing the oligomer. Thus, CD4 binding may modulate envelope glycoprotein epitopes by inducing: (*a*) an allosteric change within individual gp120 molecules; (*b*) conformational changes between adjacent molecules within an envelope glycoprotein oligomer; or (*c*) both.

Previous studies have demonstrating that the V3 loop is important for virus entry: antibodies to this region potently block virus infection without interfering with gp120/CD4 binding (37, 38, 47, 48), and mutations in this region block virus-cell fusion (49, 50). The role of this region in HIV fusion is not, however, understood. It may be that the increased exposure of the V3 loop after sCD4 binding allows its interaction with a cell surface molecule additional to CD4, such as a cell surface proteinase, for which there is inferrential in vitro evidence (43, 51–54). Alternatively, exposed V3 loops may interact with a second site on CD4, a proposal in accord with the substantial downmodulation of V3 loop staining after binding of sCD4 at 37°C (see Fig. 2 *a*).

It is generally agreed that the NH_2-terminal hydrophobic domain of gp41 plays a critical role in membrane fusion (32–36). Direct evidence of the exposure of the fusion domain of gp41 is, however, currently lacking and will require detection of this region immediately after sCD4 binding. Our results from staining HIV-infected cells with gp41 mAbs against two epitopes show that some regions of gp41 are normally exposed on infected H9 cells. This may be attributable to the exposure of gp41 molecules that have lost gp120 spontaneously, or to the partial exposure of these epitopes in the intact oligomeric gp120/gp41 complexes. The increase in staining after sCD4 binding may represent increased exposure of gp41 epitopes as a result of any or all of the following: (*a*) conformational changes in gp41-associated gp120 molecules; (*b*) conformational changes within gp41 oligomers; (*c*) dissociation of gp120/sCD4 complexes from gp41. The two epitopes on gp41 that we have studied do not correspond to regions with known function. The mAb 50-69 recognizes an immunodominant region that has previously been shown to be highly conserved among HIV-1 isolates (55), and structurally conserved in many other retroviruses. Interestingly, a peptide synthesized from a sequence directly adjacent to the 50-69 epitope binds to a 44-kD cell surface molecule, and inhibits HIV infection of $CD4^+$ cells (56). mAb 98-6 binds gp41 nearer to the COOH terminus, within a suggested α helix (57).

A rapid consequence of sCD4 binding to gp120 at 37°C is the dissociation of gp120 from gp41 (23–26). We have speculated that this may be an initial step in the membrane fusion process. It is clear from our present data that conformational changes in the viral envelope can take place without dissociation of gp120 from gp41, albeit at 4°C. Thus, complete separation of the envelope glycoprotein subunits may not be a prerequisite for the fusion reaction.

Although there are as yet no data demonstrating a direct role of CD4 in activation of the fusogenic mechanism in HIV (57), there are observations of enhanced syncytium formation and virus infectivity observed by us with certain strains of HIV-1 (Q.J. Sattentau, unpublished results) and others with SIV (29, 30) and HIV-2 (31; and P. Clapham, personal communication) when sub-inhibitory concentrations of sCD4 are added to syncytium or infectivity assays. Since such enhancement can take place with $CD4^-$ cells (P. Clapham, personal communication), it may be that the role of CD4 in activation of HIV membrane fusion is as important as its role in virus binding (58).

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